7. *Leucocytozoön musculi*, sp. n., a Parasitic Protozoön from the Blood of White Mice.

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(Plate XXXIX.† and Text-figure 154.)

CONTENTS.

I. Introduction.

The microscopic organism described in this memoir was found in the leucocytes or white blood-corpuscles of white mice. It is a parasitic Protozoön belonging to the class Sporozoa, and being a blood parasite, it is included in the order Hæmosporidia. As it occurs in leucocytes, it should be placed in the genus *Leucocytozoön*. Similar parasites have been described during the last three or four years from the leucocytes of dogs, rats, palm-squirrels, and cats among mammals, and a few from other Vertebrates. These will be briefly considered at the end of this memoir. So far as I know, such a parasite has not been recorded previously from the mouse.

As the parasite is shaped like a Gregarine, it is closely related to the genus *Hæmogregarina*, and some authorities, as Laveran and Mesnil, would place the Leucocytozoa in that genus. I prefer, however, to retain the generic name *Leucocytozoön*, and since the parasite occurs in the mouse, *Mus musculus*, I propose the specific name "*musculi*" for it. This creation of a new species is made, not with the intention of merely multiplying species, but to avoid confusion with the parasites found in rats. The nomenclature of these parasites of rats is in a very confused

* Communicated by H. B. Fantham, D.Sc., A.R.C.Sc., F.Z.S.

† For explanation of the Plate, see p. 715.

state. The specific name "*muris*" has been applied by Balfour [2, 2 A]* to the parasite he described from *Mus decumanus* at Khartoum. A similar parasite has been described by Adie [1] from *Mus rattus* in the Punjaub, under the name of *L. ratti*, while a third has been described by Cleland [7] from Perth, Western Australia.

I think it is very probable that this Leucocytozoön is different from that in rats. In this connection it is worthy of note that the Trypanosomes of rats and mice belong to different species.

The name that I propose for this parasite from the leucocytes of white mice is, then, *Leucocytozoön musculi.*

II. Materials.

The first infected animal received was a white mouse which came from a stock kept at St. Mary's Hospital Medical School. This white mouse, when in an almost comatose condition, was brought to the notice of Dr. Fantham. Some symptoms were feebleness of movement (constantly moving slowly, more or less in a circle, with a tremor or waltz, though the animal did not appear to be related to Japanese waltzing mice), partial closure of the eyes with discharge therefrom, and incontinency of urine.

A few parasites were found in the peripheral blood of this mouse, but the rodent soon died. As soon after death as possible, smears were made of the heart, spleen, kidney, liver and gut-contents. There was an extravasation of blood into the gut of the mouse and in this parasites were found. However, in the gut many Bacteria occurred, and it is not suggested that the Leucocytozoön was the sole cause of the death of the mouse.

A second white mouse, from the same stock as the first one, was procured and kept under observation for nearly three weeks. A few parasites only occurred in its peripheral blood. Accidental death overtook this mouse and immediately smears were made of its internal organs. In these parasites were seen but in much fewer numbers than in the first specimen. A third mouse from the same stock was found to be infected to a greater extent than the preceding one.

The first two mice were scarcely, if at all, verminous, but on the third one, many lice, *Hæmatopinus spinulosus*, were seen. These were dissected and examined for stages in the life-history of the parasite, as stated in the sequel.

The material was examined as far as possible in both the living and fixed condition, as the examination of living material is most important and tends to be overlooked.

The above-mentioned material was kindly given to me by Dr. Fantham.

III. Occurrence of Parasite.

The blood of the mouse contained parasites of two forms:—(1) large, vermiform organisms, free-living in the plasma; and

* The numbers in square brackets refer to the list of Literature at the end of this paper.

(2) smaller forms, which are cytozoic. At first the latter are free in the plasma, then later, they penetrate usually into mononuclear leucocytes where they feed and grow, finally assuming again the free form. Very rarely do they occur in polymorphonuclear corpuscles, but specimens were seen in transitional corpuscles.

No parasites were seen within tissue-cells, whether of liver, heart, spleen, lung, kidney or gut. They seem purely blood parasites, though their presence appears to cause enormous hypertrophy of cells in their neighbourhood. This was especially well seen in the liver smears. The parasites were most abundant in these smears and in the portal blood, were fairly numerous in heart and kidney smears, but very few occurred in either lung or spleen preparations, though the latter organ (spleen) was enlarged. Bone-marrow preparations were also made, and schizogony was found to occur therein.

In the case of the first mouse, extravasation of blood into the gut had occurred and the gut-contents showed free parasites in this blood. Live parasites were studied usually from freshly shed peripheral blood.

Examination of the organs of the lice showed vermicule stages of the parasite in the gut and Malpighian tubes.

The Leucocytozoa were never associated with Trypanosomes in these mice, though such an association has been described for other Leucocytozoa [1] [7].

The lice appear to act as mechanical agents in propagating the disease, for lice removed from the third mouse and placed on another resulted in a very slight infection of the hitherto unaffected one. In the case of *L. canis*, Gerrard [9] reported that puppies, which were placed together, were cross-infected by the agency of ticks.

IV. Methods.

(a) *Fresh material.*

Freshly drawn blood, usually taken from the tip of the tail of the mouse under examination, was mixed with a small quantity of normal saline solution, to which in most cases a little alkaline methylene-blue was added. A drop of the mixture was examined in the well of a micro-slide provided with such a depression, or else on the slide or on the cover-slip, forming a hanging drop in the latter case. The cover-glass was always vaselined round the edges and so air in quantity was excluded from the preparation.

In this way, living parasites could be observed for several hours. Intra vitam staining with methylene-blue could also be thus accomplished. Much time was spent in examining the parasites in the fresh state.

Lice found on the third mouse were carefully examined for probable stages in an Invertebrate host. Hemiptera removed from the mouse were at once dissected in normal saline solution. Especial attention was paid to the alimentary canal, Malpighian

tubules, salivary glands, reproductive organs, and body-cavity of the lice. Smears of these organs, fixed wet with osmic vapour, were afterwards stained and examined microscopically, but fresh preparations in normal saline to which a little methylene-blue had been added, were also examined in this case.

(b) *Fixed material.*

The blood smears were usually fixed wet with osmic vapour or with osmic vapour and alcohol. Occasionally they were allowed to dry quickly and afterwards treated with methyl alcohol before staining. The chief stains used were Giemsa's mixture of azur II. and eosin, Loeffler's alkaline methylene-blue, and Delafield's hæmatoxylin, the latter used alone or sometimes followed by safranin. Azur II. followed by lichtgrün was tried occasionally, and safranin alone—suggested by the presence of a refractile cyst-like envelope round some of the parasites—was tried, but found to be too transparent and diffuse. The best results were obtained with Giemsa's stain, alkaline methylene-blue, and hæmatoxylin. The same stains were used for the organ smears of lice.

The preparations were usually mounted in Canada balsam, sometimes left uncovered.

The various methods outlined above were tried for the purpose of correlation and corroboration, and to eliminate possible errors.

V. General Structure.

The general shape of this parasite, which occurs either inside a leucocyte or free in the blood-plasma, may be described as vermiform or gregariniform, in fact, that of a vermicule (Pl. XXXIX. figs. 1, 2). However, there is very often no marked difference between the ends, which are then somewhat rounded; and further, as some of the parasites are comparatively broad for their length, they may be quite accurately described as bean-shaped or reniform. The size varied from 17μ to 7μ in length and $5{\cdot}9\mu$ to 4μ in breadth.

The free parasites, averaging $10{\cdot}9\mu$ long by $5{\cdot}1\mu$ broad, are usually surrounded by a cytocyst which is very refractile and does not stain at all easily (Pl. XXXIX. fig. 8). Their cytoplasm is rich in granules which react vigorously toward stains and thereby obscure the oval nucleus lying beneath them. The distribution of the granules varies, and this accounts for the differences in the nuclear apparatus as seen in the figures. The nucleus is generally nearer one end in position.

Differences between the ends of the organism occur in cytozoic forms. One end may be larger than the other. This may be the natural result of the cytozoic habit, the organism assuming the form most suitable to a limited space, or it may be due to the twisting of the body on itself and within the spherical leucocyte, which results in the "thin edge" or "tailed" appearance of some of the parasites (text-fig. 154 D–K). **U**-shaped forms as

described by Wenyon [14] were not seen. The average size of endo-globular forms was 8μ long by 5μ broad.

Among the free forms, some are relatively shorter and broader than the others (Pl. XXXIX. figs. 1, 6). Some writers [12, 13] would consider these broader forms as female Leucocytozoa, while the longer thinner ones (Pl. XXXIX. figs. 5, 8) would be regarded as male. I have no evidence to support this view, but regard them as extreme forms of a continuous series.

VI. Movements.

(a) *Movements of Trophozoites.*

When a parasite has penetrated a leucocyte, it remains at first near the periphery and so directs its movements that it ultimately comes to surround the nucleus to a very great extent.

Osmotic diffusion between host cell and parasite occurs and produces movement within the cell. When this is very vigorous, the oscillation produced may be so great as to cause semi-rotation of the leucocyte, even to the extent of 180°.

The movement of the parasite is more noticeable at one end. This appears to advance steadily by an outflow of the cytoplasm. This outflow is easily seen, for the protoplasm is richly granular and stains readily intra vitam with methylene-blue. The parasite lies near to the nucleus of the leucocyte, and its presence causes a movement of the nucleoplasm which appears in a state of agitation. Osmosis seems to be taking place from the leucocyte nucleus to the parasite, and the latter rapidly grows during this period (text-fig. 154, A–E).

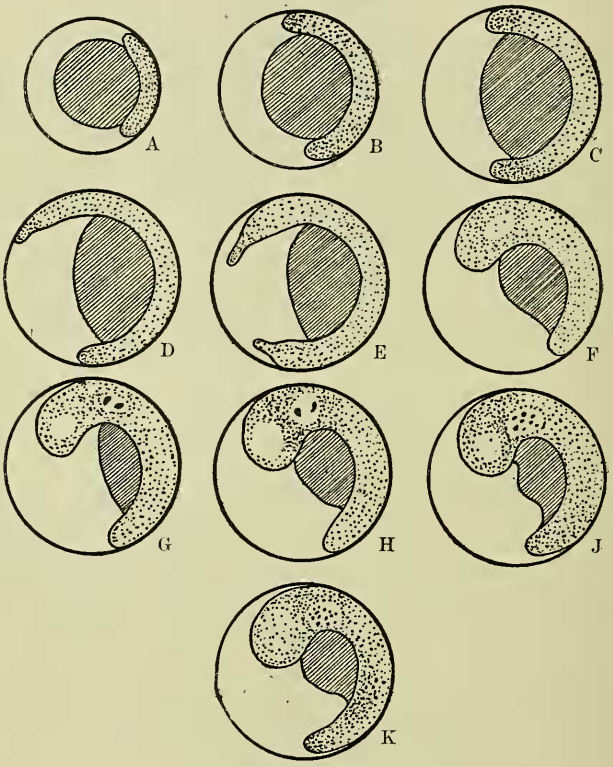
The gliding movement continues, and the nucleus of the leucocyte, which was originally globular, becomes much altered in shape. Its nuclear membrane becomes less and less distinct, and at times it resembles a somewhat lens-shaped mass lying within the horns of a crescent formed by the parasite (text-fig. 154, D, E).

The organism continues its gliding movements and one end becomes much larger than the other. This is the more obvious and may be termed the anterior end. A comma-like appearance then results (text-fig. 154, G–J). The posterior end, being thinner, might be termed a tail, though this is not an accurate description. It certainly appears very filamentous at times, but this is because the organism has turned on its side and so exposed an edge to view.

On examining the surface of the parasite, numerous granules are evident. These are usually arranged in more or less regular rows. During movement of the organism as a whole, movements of the rows occur, and this suggests that the arrangement of the granules in rows is due to myonemes upon the body. Stained preparations show that such is the case (Pl. XXXIX. fig. 7). The slow gliding movement would be due then to contractions of these myonemes, and, further, the bowed appearance of the parasite within the cell could be explained as being the result

of the stronger contractions of the myonemes at the inner edge of the organism with successively smaller contractions of the myonemes toward the outer (greater) curvature, where there would be a ring of relaxed myonemes. The axial line of the body, where, judging from the action of the granules, the movement is least, would be, according to this arrangement, a neutral area, neither contraction nor relaxation of the myonemes occurring within it.

Text-fig. 154.



Living *Leucocytozoön musculi.*

Diagrams of a young intra-corpuscular parasite observed in the living condition for seven hours.

In these diagrams the cytoplasm of the parasite is represented by small dots, more closely aggregated where the protoplasm stained more deeply. Vacuoles are left clear. The chromatin of the parasite is represented black in G–K, and the nucleus of the leucocyte is shown by shaded lines. The outline of the leucocyte is indicated by a circular area.

The nucleus of the leucocyte is not yet much displaced by the parasite, and no marked hypertrophy of the host-cell has yet occurred.

The time required for the assumption of the various forms

mentioned varies with the specimen and with the stage of development at which the parasite may be. At the initiation of the trophic, intracellular phase of the life-history, the activity of the organisms is very great, and, correlated with this, there is the phase of most vigorous motion. The infected leucocyte figured in text-figure 154, A–K, was observed for a period of seven hours, and even then the exit of the parasite from the leucocyte was not seen, owing to the death of the leucocyte.

An early stage is seen in text-figure 154, A, where the parasite was lying in the leucocyte so that the nucleus of the latter was practically in full view. About twenty-five minutes later, the organism had spread itself out and become crescentic in form (text-fig. 154, B, C). The movements till then were very vigorous, and much oscillation of granules in both parasite and leucocyte nucleus was observed. Half an hour after the observation was commenced, the forward movements of the parasite slowed very much, though the movements of the granules remained much the same (text-fig. 154, D). About the same time the protoplasm of the parasite began to move from the periphery towards the anterior end, which became much more globose and began to curve (text-fig. 154, E, F). An anterior end could be distinguished definitely as such at the end of 55 minutes (text-fig. 154, F). Two hours after the first observation was made the parasite had assumed the "comma" form and a vesicle began to make its appearance (text-fig. 154, F–J). It remained in this condition for a long time, the only change that occurred being that the protoplasm became much more granular and there was a slight retraction of the posterior end, while the vacuole increased in size and chromatin masses appeared in it (text-fig. 154, G–K). Death of the leucocyte prevented further reliable observations being made on this parasite, but the exit of the organism from its host-cell was seen in other specimens (Pl. XXXIX. figs. 18, 19).

Extrusion of the parasite is brought about by internal pressure. The Leucocytozoön moves forwards with a slow, gliding movement. This continues steadily until extrusion is completed. There is slight resistance at the periphery of the leucocyte, but on the exit of the parasite, the protoplasm of the host-cell closes up and the point of exit is invisible (Pl. XXXIX. figs. 18, 19). The freed parasite remains quiescent for a short time after leaving the corpuscle.

Stages of endoglobular parasites are figured in Pl. XXXIX. figs. 15, 16, 17.

(b) *Movements of the small, free Merozoites.*

In some of the peripheral blood mixed with normal saline to which a little methylene-blue was added, sausage- or bean-shaped bodies occurred (Pl. XXXIX. fig. 13). These moved fairly actively, the movements being much more energetic if the organism were in the neighbourhood of a leucocyte. The body of the parasite would seem to be somewhat flattened, for it is

able to turn freely somewhat in the fashion of *Nyctotherus*. The anterior end remains still, but the posterior part of the body turns over so that the upper surface becomes folded over the under, which, at the distal end, is now uppermost. The line of folding is somewhat oblique. Reversal of this movement occurs, and the organism appears to roll from side to side as a result of the combined movements.

Accompanying this movement there is a second. One surface of the body of the parasite contracts, and as a result, the ends of the body approximate somewhat more closely to one another than before. Relaxation follows, and the body straightens with a jerk which has the effect of forcibly propelling the organism forwards.

The path of the organism is never straight. Movement appears to be initiated at either end indifferently. The path is often very restricted and the organism remains for long periods at practically the same spot, though one of its ends may have vibrated in practically every direction.

VII. Detailed Morphology.

(a) *The Trophozoite.*

Ectoplasm.—In the trophozoite this is not markedly differentiated. It appears as a somewhat clearer portion in some specimens. Contractile elements or myonemes are present, arranged in longitudinal rows. These myonemes are very evident in some specimens (Pl. XXXIX. figs. 7, 11) and often are well seen in the region of the nucleus. A refractile cytocyst is often present, and when this is well marked, a clear space usually intervenes between it and the ectoplasm (Pl. XXXIX. figs. 8, 9, 10, 11, 12). The cytocyst is always thin and membranous.

Endoplasm.—This is richly granular (Pl. XXXIX. figs. 1, 4) and the granules react vigorously towards stains. In some specimens the endoplasm appears almost alveolar (Pl. XXXIX. fig. 12) owing to the disposition of the granules in regular networks. There are concentrations of granules beneath the myonemes and also in the region of the nuclear membrane. Frequently a relatively clear space appears near one end, almost suggestive of a large vacuole (Pl. XXXIX. figs. 3, 4, 7, 21). In a few specimens isolated chromatic granules are present (Pl. XXXIX. fig. 8). The latter do not seem to have any direct connection with the nucleus.

Nucleus.—This is circular or oval, approximately central in position or sometimes nearer to one end and possesses a definite nuclear membrane. Its chromatin is diffusely spread within and much achromatic substance is present. The structure of the nucleus may best be described as vesicular. There does not appear to be a karyosome as described by James [**10**] in one of his forms of *Leucocytozoön canis*. In its general structure and behaviour towards stains, the nucleus of this parasite is very suggestive of that of *Trypanosoma rajæ*.

The nuclear membrane is definite. It has a somewhat beaded appearance in some stained preparations and that can also be seen in living material (Pl. XXXIX. figs. 2, 4, 6, 16). Extra-nuclear chromatin also occurs, for in favourably stained specimens, a chromatic cap can be distinguished at either end (Pl. XXXIX. fig. 15), and isolated granules also occur (Pl. XXXIX. fig. 8).

(b) *The vermicules of* Hæmatopinus spinulosus.

The vermicules of the lice present the following appearance:—

Their ectoplasm is not well differentiated from the endoplasm, but indications of myonemes are present and are best seen at the ends of the organism.

The endoplasm is granular and much as in the trophozoites, but marked concentrations of granules do not occur.

The nucleus in some appears to lie in a vacuole, and in most specimens there is a clearer portion in the neighbourhood of the nucleus. Its chromatin is more abundant than in the blood-inhabiting forms, and the nuclear membrane is fairly distinct (Pl. XXXIX. figs. 23, 24).

These vermicules were about $8{\cdot}8\mu$ long and $1{\cdot}4\mu$ broad.

VIII. Multiplication.

(a) *Schizogony.*

Examination of bone-marrow showed the presence of small, oval cysts (cytocysts) about $13{\cdot}1\mu$ long by $8{\cdot}9\mu$ broad (Pl. XXXIX. fig. 20). These contained relatively few (about 12) but distinct, small forms with a definite vermicular or reniform contour. Each of these small vermicules was about $4{\cdot}4$ long by $\cdot 8\mu$ broad (Pl. XXXIX. fig. 20). They are the merozoites, produced by the multiple or asexual fission of a schizont inside the cytocyst. The general protoplasm was granular. Remains of the nucleus of the leucocyte host were seen on the side of the cyst. Inside the cyst were also some remains of the residual protoplasm of the schizont. By the dehiscence of the cyst these merozoites are set free in the blood-plasma, where they become vermicules or young trophozoites.

(b) *Possible Association of Trophozoites.*

Two parasites lying in one corpuscle were observed (Pl. XXXIX. fig. 21), or two which had just left the corpuscles (Pl. XXXIX. fig. 22). One such case, of two parasites lying in the remains of a leucocyte, suggested possible association (Pl. XXXIX. fig. 21). Here the two forms, partially free from the host-cell or leucocyte, came in contact with one another and became enveloped in a common cytocyst. The nucleus of one appeared to come nearer the area common to the two than the other, and a chromatin mass was seen in the common area of the couple. This suggested that transference of chromatin takes place from one parasite to the

other. However, the formation of a definite zygote from these associated forms was, unfortunately, not seen.

Again, in Pl. XXXIX. fig. 22, those authorities who believe in differentiation into male and female forms, would see a female form (macrogametocyte) in the upper, broader and granular parasite, and a male form (microgametocyte) in the lower, longer and narrower form. I do not go quite so far personally, as I have doubts of sexual differentiation, preferring the series view (see page 707). However, in the present state of our knowledge, the suggestion of sexual forms is worthy of note.

IX. Life-History.

Commencing with the free vermicule which may be either a sporozoite or a merozoite, the following sequence probably occurs. The parasite lives awhile in the plasma as a small, active form (Pl. XXXIX. fig. 13). This ultimately penetrates a leucocyte and grows actively there as an endoglobular trophozoite. A cytocyst may or may not form around it (Pl. XXXIX. figs. 15, 16, 17). After a time, it becomes free in the plasma, rupturing the host-cell as it issues, but leaving little or no trace of its presence behind. In the plasma it assumes the free trophozoite form. Association between trophozoites may perhaps occur (*cf.* Pl. XXXIX. figs. 21, 22), and the result of this is probably a zygote, which one would expect to find in the louse, on homology with the malarial parasite—but more definite information is lacking.

Other free forms may reach the bone-marrow. There encystment occurs, and a schizont, in a thin but distinct cytocyst, is produced. This schizont by multiple fission gives rise to relatively few but definite merozoites. By the rupture of the cyst, these are set free into the blood-stream where growth again occurs, leading to adult trophozoites. This is the schizogonic cycle of the parasite.

Ecto-parasitic on the mouse was the louse, *Hæmatopinus spinulosus*. By the bite of this louse infected blood passes from the mouse to the mouth and gut of the invertebrate host, and so we find the small, gregariniform vermicules shown in Pl. XXXIX. figs. 23, 24. These ultimately reach the saliva of the louse and by this insect are probably transferred to another mouse. Perhaps a sexual cycle of the Leucocytozoön occurs in the louse, but of this I have, unfortunately, no definite evidence. Probably the louse is merely a mechanical agent in spreading the infection.

X. Affinities of the Parasite, and Summary.

Affinities of Parasite.

The term Leucocytozoön was used by Danilewsky [8] in 1890 for vermiform parasites stated to occur in the leucocytes of certain birds. Danilewsky confined his observations to fresh preparations.

Similar parasites were afterwards studied by Berestneff, Sacharoff, Ziemann, and Laveran. The latter states that Danilewsky's parasites really occur in immature erythroblasts. The parasite was stated later by Schaudinn [12] in 1905 to be a stage in the life-history of a Spirochæte. The matter is fully discussed by James [10], and as it is very controversial, need not be dwelt on further here.

Bentley [3] and James [10] independently described a parasite from the leucocytes of pariah dogs in India in 1905. This was a true Leucocytozoön and is known as *L. canis*. A memoir by Christophers [5] on the same parasite appeared in 1906, and the following year he worked out the sexual cycle in the tick [6]. Other Leucocytozoa have been described in mammals by Patton [11] in the Indian palm-squirrel, *Funambulus pennantii*, under the name of *L. funambuli*; in Malay dogs by Gerrard [9] and Wenyon [14]; and in rats in various parts of the world by Balfour [2] from leucocytes of *Mus decumanus* at Khartoum, by Adie [1] from *Mus rattus* in the Punjaub (as *L. ratti*), and by Cleland [7] from rats in Perth, Western Australia. These parasites in rats are probably best known as *L. muris* (cf. page 704). A form known as *L. felis* has been described, I believe, from the Indian bazaar cat in Madras by Christophers and Patton.

Outside mammals, from other vertebrates, we have recorded *L. ranarum* from the Amphibian *Leptodactylus ocellatus* by Carini [14]; and quite recently *L. lovati*, a form from the leucocytes of grouse by Seligmann and Sambon [13].

These are, I think, all or nearly all the Leucocytozoa recorded to date (May, 1908). Whether they have any intimate connection with Flagellates, such as that suggested by Schaudinn [12] remains to be seen—probably they have not.

These parasites are all gregariniform. Those in mammals are much about the same size, and occur both free in the plasma or endoglobular in the leucocytes. Schizogony is known in the bone-marrow [5] and liver.

Laveran suggested that the Leucocytozoa should be included in the genus *Hæmogregarina*. However, as they occur in a non-hæmoglobin-containing host, viz. leucocytes, while strict Hæmogregarines are found in erythrocytes, I think the generic name *Leucocytozoön* should be retained. The name for the parasite described in this memoir for the first time in the leucocytes of the mouse is *L. musculi*.

Summary.

(1) The forms of *L. musculi* here described occur in the mononuclear and transitional leucocytes of white mice.

(2) The free trophozoites in the plasma are gregariniform or reniform vermicules, the average size being 10·9μ long and 5·1μ broad (*cf.* Pl. XXXIX. figs. 1–12).

(3) The free trophozoites sooner or later enter leucocytes of the host and grow at the expense of the nucleus of the leucocyte

(*cf.* *Karyolysus* among the Hæmogregarines). A thin cytocyst is formed probably by the host-cell around the parasite (Pl. XXXIX. figs. 16, 17).

(4) Endoglobular forms are on the average 8μ long by 5μ broad (*cf.* Pl. XXXIX. figs. 15, 16, 17).

(5) Schizogony takes place in the bone-marrow. An endoglobular trophozoite rounds itself off and becomes a schizont, breaking up into merozoites, each about 4·4μ long and ·8μ broad (Pl. XXXIX. fig. 20). This again suggests affinities with *Karyolysus*, judging by Labbé's figures of schizogony in *Karyolysus*.

(6) Two parasites may sometimes occur within one host-cell. Two such forms suggesting the beginning of association are shown in Pl. XXXIX. figs. 21, 22.

Differentiation into male and female forms could not be made out with certainty, though some parasites were shorter, broader, and more granular than others.

(7) Vermicules were found in the gut and Malpighian tubules of lice, ectoparasitic upon the mice, but unfortunately no evidence of a sexual cycle in the louse was obtainable. Perhaps the lice merely act as mechanical agents in the transfer of the parasites among the mice.

(8) The parasites are found in smears from the heart and liver in abundance. They are less numerous in spleen and kidney smears, also in the bone-marrow and peripheral circulation. They were not abundant in the latter.

(9) No Trypanosomes were seen in the infected mice.

(10) The movements of the vermicules or trophozoites of this parasite in the blood-plasma of its Vertebrate host are fully described in section VI. of this memoir.

XI. References to Literature.

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XII. EXPLANATION OF PLATE XXXIX.

The figures on the Plate XXXIX. were outlined with camera lucida (Abbé), using Zeiss $\frac{1}{12}$ inch achromatic and 2 mm. apochromatic oil-immersion objectives with compensating oculars 4 and 8. Zeiss E objective was also used for fresh preparations.

Fig. 1. Shows a free trophozoite which is broad and short. Stained Giemsa. × 1700. Liver smear of 1st mouse.

Fig. 2. Free trophozoite, rather large. Shows one chromosome. Giemsa. × 1700. Liver smear of 2nd mouse.

Fig. 3. Smaller, possibly younger, form. It has one patch of chromatin. Giemsa. × 1700. Liver smear of 1st mouse.

Fig. 4. Form showing dotted nucleus, also dots along the nuclear membrane. Giemsa. × 1700. Heart smear of 1st mouse.

Fig. 5. Parasite showing central chromatin filaments. The Leucocytozoön is not so markedly granular as some. Giemsa. × 1700. Liver smear of 1st mouse.

Fig. 6. *Cf.* fig. 1. The nucleus is vesicular and chromatin is distributed round the nuclear membrane. Giemsa. × 1700. Liver smear of 2nd mouse.

Fig. 7. Shows pale, vesicular nucleus, also myonemes (*my*) are well seen. The ends are somewhat pointed. Giemsa. × 2250. Liver smear of 2nd mouse.

Fig. 8. Parasite in a clear cytocyst (*cy*). It has a patch of extra-nuclear chromatin in the endoplasm. Giemsa. × 1700. Liver smear of 1st mouse.

Fig. 9. Parasite in cytocyst. Caps of chromatin present. Giemsa. × 1700. Kidney smear of 1st mouse.

Fig. 10. Parasite in cytocyst with deeper staining nucleus; also very granular protoplasm. Giemsa. × 1700. Liver smear of 1st mouse.

Fig. 11. Shows pale-staining vesicular nucleus and definite myonemes (*my*). In cytocyst (*cy*). Giemsa. × 1700. Liver smear of 1st mouse.

Fig. 12. Broad parasite within cytocyst (*cy*), showing alveolar protoplasm. Giemsa. × 1700. Spleen smear of 1st mouse.

Fig. 13. Free forms in plasma. Methylene-blue. Peripheral blood of 3rd mouse.

Fig. 14. Free vermicule penetrating mononuclear leucocyte. Methylene-blue. × 1200. Peripheral blood of 3rd mouse.

Fig. 15. Young intra-corpuscular stage. Parasite has a chromatin cap at either end. Giemsa. × 1700. Liver smear of 2nd mouse.
Figs. 16, 17. Endoglobular parasites in mononuclear Leucocytes. Giemsa. × 1700. Liver smear of 1st mouse.
Fig. 18. Shows egress of parasite from its host-cell. Intra vitam staining with methylene-blue. × 1200. Peripheral blood of 3rd mouse.
Fig. 19. Egress of parasite shown in fig. 18 from leucocyte just completed, after rotation of leucocyte. Intra vitam. Methylene-blue. Peripheral blood of 3rd mouse.
Fig. 20. Schizont within cytocyst in bone-marrow. Twelve merozoites are present, together with residual protoplasm (*r.pl.*). Methylene-blue. × 1600. Bone-marrow of 2nd mouse.
Fig. 21. Possible association of the two Leucocytozoa. A common cytocyst is present. Chromatin is showing passing across from one to the other. One Leucocytozoön is not entirely free from its host-cell. Each of the couple has a vacuole. Giemsa. × 1700. Liver smear of 1st mouse.
Fig. 22. Possible association. Both of the Leucocytozoa are free from their host-cell or cells. Liver smear of 1st mouse. Giemsa. ×1700.
Figs. 23, 24. Vermicules from the midgut of *Hæmatopinus spinulosus*. Giemsa. × 1700.

8. Descriptions of African Micro-Lepidoptera.

By E. Meyrick, B.A., F.R.S., F.Z.S.

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This paper is a contribution towards the knowledge of the Tortricina and Tineina of the African region, which are at present very little known. The material for it was received from various collectors, but for the greater part of it I am indebted to Mr. A. J. T. Janse, of Pretoria, whose specimens are also particularly notable for their fine condition. Mr. Janse further furnished notes on localities and time of appearance, based on additional examples retained by himself besides those which he sent me. In addition to some known species recorded from Africa for the first time, 108 species and 10 genera are described as new.

Eucosmidæ.

Lobesia aeolopa Meyr.

This species, described from India and Ceylon (Journ. Bomb. N. H. Soc. xvii. p. 976), I possess also from Grahamstown, Cape Colony, and the island of Réunion.

Polychrosis harmonia, sp. n.

♂ ♀. 10–13 mm. Head, palpi, and thorax light ochreous, face whitish-suffused. Abdomen whitish-ochreous, sometimes suffused with grey. Fore wings elongate, slightly dilated posteriorly, costa slightly arched, apex obtuse, termen obliquely rounded; pale brownish-ochreous; markings deep yellow-ochreous; an inwardly oblique spot beneath fold before middle (representing lower portion of angle of a strongly angulated basal patch, of which remainder is obsolete); central fascia rather narrow, sometimes with a few